

Kansas State Agricultural College.

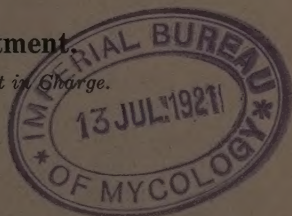
EXPERIMENT STATION.—Bulletin 161.

ED. H. WEBSTER, *Director.*

TECHNICAL BULLETIN.

Bacteriology Department.

WALTER E. KING, *Bacteriologist in Charge.*



THE INFLUENCE OF DEPTH OF CULTIVATION UPON SOIL BACTERIA AND THEIR ACTIVITIES.

BY

WALTER E. KING, *Bacteriologist.*

CHARLES J. T. DORYLAND, *Assistant in Soils.*

MANHATTAN, KANSAS,

AUGUST 12, 1909.

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The Influence of Depth of Cultivation upon Soil Bacteria and Their Activities.

By

WALTER E. KING, *Bacteriologist.*

CHARLES J. T. DORYLAND, Assistant in Soils.

INTRODUCTION.

THERE are those who believe that future work in soil bacteriology will have considerable bearing upon the problem of maintaining soil fertility. This belief is founded upon the existing knowledge of soil bacteria, and the suggested part that bacterial action plays in preparing the soil for plant growth.

Granting that our present rather limited knowledge portends to show that soil bacteria and their activities are useful and essential attributes of fertile soil, it seems justifiable that attempts should be made to determine the influence of different conditions upon the number and specific activities of the bacteria living in various types of soil.

The consideration of the influence of depth of cultivation upon the soil bacteria and their activities is a broad subject, which embraces a host of minor problems. The whole problem can be determined only by an immense amount of experimental work and years of patient labor. The experiments outlined in the following pages, therefore, must be considered as preliminary and the suggested results as only forerunners of definite conclusions.

Part I.

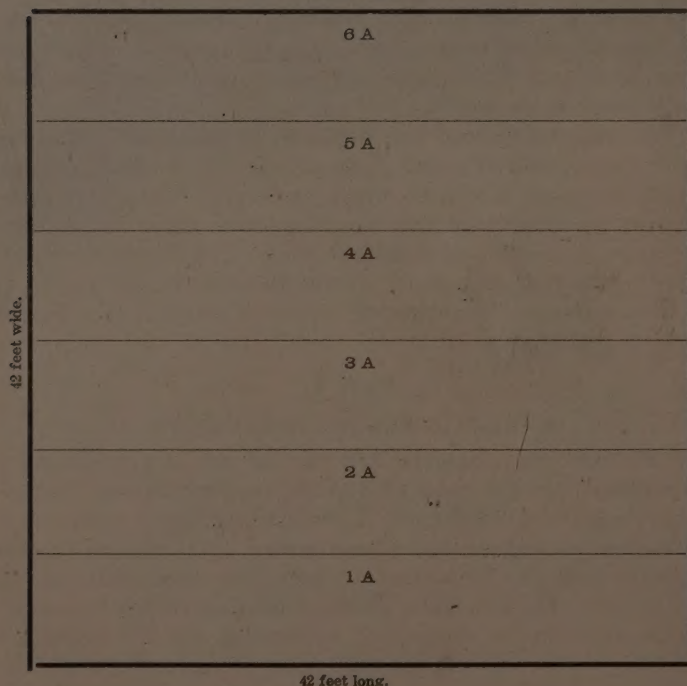
PRELIMINARY EXPERIMENTS.

This work was designed and carried out as a preliminary experiment for the basis of further research in soil bacteriology under field conditions. The work was begun on March 7, 1908, and was continued fifteen weeks. At the end of this time the plan was broadened out, including more details of soil conditions. The first three weeks of the experiment were used in determining the number of bacteria in the soil under the existing conditions, so that the comparative results by differ-

ent treatments must be drawn from the last twelve weeks' work.

The study included two types of soil as nearly opposite in character as it was possible to secure. Silt loam and fine sandy loam were selected. The silt loam is situated upon the upland Kansas State Agricultural College farm. This had previously been used for an experimental plot; the year previous it was planted to corn but received no special treatment or dressing. The sandy loam is located on the Kaw river bottom close to the stream; it had previously been used for general farming. The fall before this experiment was commenced it had received a liberal dressing of straw manure. A plot 42 x 42 feet was laid out on each field. The one on silt loam was called B; the one on sandy loam was called A. These plots were subdivided into six smaller plots, and called 1B, 2B, 3B, etc. Those of A were likewise named. The following diagram illustrates the plan of each plot:

PLOT A.



Subplots 1A and 1B remained in the original condition and received no treatment, except to keep the weeds down. This was done by shaving them off at the surface of the ground with a hoe. The remainder of the subplots, from 2 to 6 of both A and B, were stirred to a depth of two inches after every rain, as soon as the soil was dry enough to till. Subplots 2, 3, 4, 5 and 6, of both A and B, were plowed to a depth of two, four, six, eight and ten inches, respectively.

In order to determine the influence of the different depths of cultivation on the number of bacteria, six samples were taken from each subplot every seventh day. These samples were taken every second inch to the depth of twelve inches. The estimates are given as so many million bacteria per cubic centimeter. The high bacterial content obtained in some instances is perhaps due to the methods used in this work. The volumetric method of collection and preparation of samples was used exclusively.

At the same time that samples were taken for bacterial determination, samples were also taken to determine the per cent. of moisture in the surface foot of the soil. Each plot was provided with a thermometer, so that the temperature could be recorded each time the samples were taken. The results are given in the following tables (I to XII) :

TABLE I—PLOT A. Check plot, undisturbed.—Fine sandy loam (U. S. Soil Survey). Number of bacteria given as millions per cubic centimeter.

DATE.	Plot No.	Temp., C.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 19①	1A	10.0	20.17	9.70	10.92	8.64	8.82	4.74	2.88
" 25	1A	10.0	20.10	11.16	14.26	13.68	8.00	5.40	2.52
Apr. 2	1A	10.5	20.10	12.00	15.00	6.60	6.60	6.00	2.40
" 9	1A	11.0	20.25	15.00	10.80	8.40	6.00	4.20	3.00
" 16	1A	14.9	20.30	11.40	10.92	16.80	10.80	18.00	5.46
" 23	1A	17.0	24.50	30.06	12.06	4.44	3.60	1.20	1.20
" 30	1A	16.6	24.50	16.20	18.00	4.20	3.20	1.20	1.20
May 6	1A	17.5	24.70	15.00	8.40	8.40	4.20	4.80	4.20
" 13	1A	19.7	25.00	x	x	x	x	x	x
" 20	1A	16.6	25.40	4.79	5.38	3.58	4.18	2.38	.59
" 27②	1A	18.6	27.20	13.72	lost	5.32	2.40	5.32	1.10
Jun. 2	1A	20.0	27.90	22.00	18.40	6.40	4.60	7.40	3.80
" 9	1A	20.5	25.20	12.60	7.20	6.00	4.20	1.80	.60
Totals.....				173.63	181.34	92.46	66.60	62.44	28.95
Averages.....				14.47	11.94	7.71	5.55	5.20	2.41

1. Determinations made on March 19 indicate number of bacteria at beginning of experiment.

2. Samples taken after field had been saturated for several days.

Moisture estimates indicate per cent. average in first foot, one determination being made for all plots.

In all cases "x" indicates a spreading growth on plate cultures which prevented counting of colonies.

TABLE II—PLOT 2A. Stirred to the depth of two inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	2A	10.0	20.17	13.32	20.88	10.08	10.00	1.98	6.84
Apr. 2....	2A	10.5	20.10	14.40	18.00	18.00	12.60	2.40	2.40
" 9....	2A	11.0	20.25	11.40	12.00	9.36	8.70	7.20	4.80
" 16....	2A	14.9	22.80	10.80	8.46	4.26	4.80	1.20	1.80
" 23....	2A	17.0	24.60	11.40	9.00	29.52	13.20	18.60	4.26
" 30....	2A	16.6	24.50	4.20	6.60	12.00	7.80	5.40	2.40
May 6....	2A	17.5	24.70	16.40	12.40	9.20	9.20	6.80	7.60
" 13....	2A	19.7	25.00	x	x	x	x	x	x
" 20....	2A	16.6	25.40	2.98	2.98	3.58	1.18	1.18	.68
" 27....	2A	18.6	27.20	15.12	13.50	7.72	2.92	1.72	2.32
Jun. 2....	2A	20.0	27.90	11.80	17.20	10.00	1.00	1.60	2.20
" 9....	2A	20.5	25.20	7.20	6.80	5.60	2.80	3.20	1.20
Totals.....				119.02	127.82	119.20	74.20	51.28	36.50
Averages.....				10.82	11.62	10.84	6.74	4.66	3.31

TABLE III—PLOT 3A. Stirred to depth of four inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	3A	(See plot 2A)....		34.20	42.80	19.80	17.28	8.28	4.68
Apr. 2....	3A	" " " " " "		9.00	16.20	16.20	14.40	6.60	3.00
" 9....	3A	" " " " " "		15.00	28.20	①61.00	10.80	6.00	5.40
" 16....	3A	" " " " " "		4.80	4.26	9.00	3.66	1.92	1.92
" 23....	3A	" " " " " "		20.40	22.32	11.46	9.00	1.62	5.52
" 30....	3A	" " " " " "		9.00	2.60	8.40	2.40	2.40	.60
May 6....	3A	" " " " " "		34.80	35.20	11.20	2.52	2.80	6.40
" 13....	3A	" " " " " "		x	x	x	x	x	x
" 20....	3A	" " " " " "		6.58	5.98	6.00	8.98	4.78	0.68
" 27....	3A	" " " " " "		16.72	13.72	10.10	10.12	4.10	1.10
Jun. 2....	3A	" " " " " "		14.20	22.60	9.40	5.80	4.00	3.80
" 9....	3A	" " " " " "		4.80	4.80	6.00	4.00	1.20	.40
Totals.....				169.50	198.68	168.56	88.96	43.70	33.40
Averages.....				15.40	18.06	15.32	8.08	3.97	3.03

1. Bunch of manure is responsible for large number of bacteria.

TABLE IV—PLOT 4A. Stirred to depth of six inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	4A	(See plot 2A.)		11.04	11.28	8.16	8.16	5.23	4.00
Apr. 2....	4A	" " " " " "		12.00	18.60	27.40	6.60	4.20	3.40
" 9....	4A	" " " " " "		9.00	12.60	14.40	16.80	9.60	5.40
" 16....	4A	" " " " " "		8.40	19.32	7.32	6.60	3.72	1.92
" 23....	4A	" " " " " "		10.32	42.60	39.66	16.32	7.80	3.00
" 30....	4A	" " " " " "		3.00	6.00	6.00	2.40	1.80	.60
May 6....	4A	" " " " " "		14.80	15.60	13.60	2.20	1.60	2.80
" 13....	4A	" " " " " "		x	x	x	x	x	x
" 20....	4A	" " " " " "		5.98	7.78	①20.98	2.38	3.58	.98
" 27....	4A	" " " " " "		16.72	15.52	10.10	6.50	2.32	5.82
Jun. 2....	4A	" " " " " "		11.60	15.60	8.00	5.20	.60	2.80
" 9....	4A	" " " " " "		3.60	7.60	6.40	4.80	.80	.05
Totals.....				106.46	172.50	162.02	77.96	41.30	30.27
Averages.....				9.67	15.68	14.73	7.08	3.75	2.75

1. Bunch of manure is responsible for large number of bacteria.

TABLE V—PLOT 5A. Stirred to a depth of eight inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at certain depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	5A	(See plot 2A)....		7.92	9.12	6.72	6.43	4.56	3.84
Apr. 2....	5A	"	"	12.40	26.40	①42.00	12.00	7.00	6.60
" 9....	5A	"	"	8.40	8.46	8.40	12.00	6.00	6.00
" 16....	5A	"	"	3.72	12.06	9.72	10.32	3.00	3.60
" 23....	5A	"	"	30.00	16.32	12.84	8.58	7.26	9.06
" 30....	5A	"	"	4.20	2.40	6.00	12.60	6.12	.62
May 6....	5A	"	"	8.88	14.00	15.60	30.80	6.80	6.00
" 13....	5A	"	"	x	x	11.98	x	x	x
" 20....	5A	"	"	19.18	8.40	x	13.78	2.98	3.58
" 27....	5A	"	"	6.00	9.00	17.32	7.70	lost	7.70
Jun. 2....	5A	"	"	11.20	4.60	11.80	19.20	2.00	12.00
" 9....	5A	"	"	9.60	6.80	12.60	5.60	.40	1.20
Totals.....				121.50	117.56	154.98	139.06	46.12	60.20
Averages.....				11.04	10.68	14.09	12.64	4.61	5.47

1. The large number of bacteria is due to manure.

TABLE VI—PLOT 6A. Stirred to a depth of ten inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	6A	(See plot 2A)....		9.12	10.80	7.92	8.16	13.20	1.44
Apr. 2....	6A	"	"	6.00	7.20	14.40	5.40	4.00	3.60
" 9....	6A	"	"	7.20	10.80	9.60	11.40	11.40	8.40
" 16....	6A	"	"	4.20	10.20	6.12	①.....	16.20	5.52
" 23....	6A	"	"	6.72	10.20	10.80	18.75	10.92	7.20
" 30....	6A	"	"	6.00	9.00	3.60	3.00	5.40	6.60
May 6....	6A	"	"	①.....	6.80	11.20	15.60	5.20	4.80
" 13....	6A	"	"	x	x	x	x	x	x
" 20....	6A	"	"	9.58	10.78	16.18	7.78	12.58	5.98
" 27....	6A	"	"	12.50	lost	16.10	lost	lost	8.32
Jun. 2....	6A	"	"	4.00	5.60	4.40	8.80	4.60	1.60
" 9....	6A	"	"	8.00	8.80	11.20	4.40	2.40	5.60
Totals.....				73.32	90.18	111.52	83.29	85.90	59.06
Averages.....				7.33	9.01	10.13	9.27	8.59	5.87

1. So many colonies that they could not be counted.

TABLE VII—PLOT B. Check plot, undisturbed.—Preliminary experiment—Marshall silt loam (U. S. Soil Survey). Numbers indicate millions of bacteria per cubic centimeter.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 19....	1B	10.0	21.87	7.26	8.96	11.66	4.21	2.97	2.65
" 25....	1B	9.2	21.87	9.08	13.04	9.36	5.04	2.52	1.62
Apr. 2....	1B	10.0	22.60	4.30	4.90	7.30	1.80	4.20	1.20
" 9....	1B	13.0	22.25	9.00	18.60	2.00	2.40	1.20	2.40
" 16....	1B	16.0	23.80	7.20	12.00	16.20	8.40	2.40	.96
" 23....	1B	14.9	24.60	10.20	4.92	5.40	1.20	3.06	1.30
" 30....	1B	14.0	25.50	12.24	15.84	4.92	3.60	3.60	1.80
May 6....	1B	12.4	25.70	9.61	9.60	8.52	9.12	6.60	2.40
" 13....	1B	15.6	25.90	x	x	x	x	x	x
" 20....	1B	19.4	25.40	7.80	8.41	4.20	x	.30	.09
" 27....	1B	19.0	28.20	10.00	7.60	1.60	1.10	.30	.16
Jun. 2....	1B	18.0	28.90	6.40	8.80	1.00	.60	.30	.60
" 9....	1B	20.0	26.80	25.20	11.40	9.60	10.20	3.60	2.40
Totals.....				118.29	124.07	81.76	47.67	31.05	17.48
Averages.....				9.85	10.34	6.81	4.33	2.58	1.45

TABLE VIII—PLOT 2B. Stirred to depth of two inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria at different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	2B	9.2	21.87	9.02	6.72	4.32	2.88	2.88	3.24
Apr. 2....	2B	10.0	22.60	6.10	5.40	3.60	2.40	1.80	1.20
" 9....	2B	13.0	22.25	18.60	7.20	7.20	4.80	2.40	.60
" 16....	2B	15.0	23.80	10.80	10.20	15.00	12.00	12.00	7.20
" 23....	2B	14.9	24.60	8.64	7.80	1.20	3.06	.60	.50
" 30....	2B	14.0	25.50	6.90	52.20	6.20	3.18	.66	1.86
May 6....	2B	12.4	25.70	12.60	9.00	10.20	5.40	9.18	6.12
" 13....	2B	15.6	25.90	x	x	x	x	x	x
" 20....	2B	19.4	25.40	8.40	2.41	1.20	.66	.12	.05
" 27....	2B	19.0	28.20	4.00	4.70	.70	.70	1.20	.40
Jun. 2....	2B	18.0	28.90	13.60	14.80	1.00	1.00	.50	.80
" 9....	2B	20.0	26.80	15.60	11.40	6.60	4.20	3.60	1.80
Totals.....				114.26	131.83	57.22	40.28	34.94	23.77
Averages.....				10.38	11.99	5.20	3.66	3.17	2.16

TABLE IX—PLOT 3B. Stirred to depth of four inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria in different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	3B	9.2	21.89	8.64	6.96	2.53	2.18	1.88	3.24
Apr. 2....	3B	10.0	22.60	8.60	12.60	8.40	1.80	1.30	6.60
" 9....	3B	13.0	22.25	7.20	12.60	5.40	4.60	4.60	2.90
" 16....	3B	15.0	23.80	9.72	10.32	21.12	20.40	20.40	9.60
" 23....	3B	14.9	24.60	10.20	6.00	1.80	1.80	1.80	1.86
" 30....	3B	14.0	25.50	10.20	12.78	13.26	3.12	3.12	1.20
May 6....	3B	12.4	25.70	9.18	9.72	6.00	6.60	6.60	9.00
" 13....	3B	15.6	25.90	x	x	x	x	x	x
" 20....	3B	19.4	25.40	4.80	6.60	1.80	.27	.27	.21
" 27....	3B	19.0	28.20	10.70	8.90	10.70	1.20	1.20	.10
Jun. 2....	3B	18.0	28.90	24.40	11.60	2.20	.20	.20	.10
" 9....	3B	20.0	26.80	17.40	16.80	2.40	9.00	9.00	6.60
Totals.....				121.94	114.88	75.61	51.17	50.37	35.41
Averages.....				11.08	10.44	6.87	4.65	4.53	3.21

TABLE X—PLOT 4B. Stirred to a depth of six inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria in certain depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25....	4B	9.2	21.87	4.36	4.32	12.96	6.04	3.60	3.60
Apr. 2....	4B	10.0	22.60	5.40	7.80	9.00	1.80	1.80	1.20
" 9....	4B	13.0	22.25	12.00	17.40	19.20	4.20	4.80	1.20
" 16....	4B	15.0	23.80	8.64	15.12	13.60	12.06	9.06	①16.26
" 23....	4B	14.9	24.60	6.12	3.60	4.32	.60	.48	1.14
" 30....	4B	14.0	25.50	9.00	5.10	4.32	5.43	3.00	.59
May 7....	4B	12.4	25.70	6.72	4.80	8.90	8.60	6.78	4.32
" 14....	4B	15.6	25.90	x	x	x	x	x	x
" 21....	4B	19.4	25.40	14.40	8.40	3.00	.44	.43	.39
" 28....	4B	19.0	28.20	10.20	6.60	3.50	3.60	.16	.36
Jun. 3....	4B	18.0	28.90	16.80	15.90	17.20	1.60	.40	.20
" 10....	4B	20.0	26.80	17.40	9.00	9.60	5.40	3.00	6.60
Totals.....				108.04	98.04	106.60	49.67	33.51	35.86
Averages.....				9.82	8.91	9.69	4.51	3.04	3.26

1. No adequate reason can be given for the exceptionally high number.

TABLE XI—PLOT 5B. Stirred to depth of 8 inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria in different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25...	5B	9.2	21.87	8.82	8.20	8.64	8.52	8.28	2.52
Apr. 2....	5B	10.0	22.60	6.00	7.20	7.40	3.00	2.40	1.20
" 9.....	5B	13.0	22.25	18.00	19.80	9.00	4.20	3.60	.60
" 16.....	5B	15.0	23.80	7.26	13.32	13.20	4.26	3.60	7.80
" 23.....	5B	14.9	24.60	7.20	4.20	4.80	1.80	3.50	4.20
" 30.....	5B	14.0	25.50	16.32	18.60	14.84	9.20	.80	1.20
May 6.....	5B	12.4	25.70	10.32	9.00	11.40	10.30	7.20	6.60
" 14.....	5B	15.6	25.90	x	x	x	x	x	x
" 21.....	5B	19.4	25.40	x	x	6.00	9.60	.16	.23
" 28.....	5B	19.0	23.20	14.80	8.30	7.70	2.30	1.70	1.10
Jun. 3.....	5B	18.0	23.90	4.00	19.60	10.60	2.00	.10	.90
" 10.....	5B	20.0	26.80	9.00	27.00	12.60	4.80	4.20	1.20
Totals.....				101.22	136.22	106.18	59.98	40.54	27.55
Averages.....				10.12	13.52	9.65	5.45	3.68	2.50

TABLE XII—PLOT 6B. Stirred to a depth of ten inches.

DATE.	Plot No.	Temp.	Per cent. water.	Number of bacteria in different depths.					
				2 in.	4 in.	6 in.	8 in.	10 in.	12 in.
Mar. 25...	6B	9.2	21.87	2.88	3.60	19.64	2.88	2.52	1.42
Apr. 2....	6B	10.0	22.60	2.40	8.40	3.20	3.60	14.40	2.40
" 9.....	6B	13.0	22.25	7.20	6.60	7.80	7.90	7.20	6.60
" 16.....	6B	15.0	23.80	10.20	24.60	27.00	12.06	12.00	12.00
" 23.....	6B	14.9	24.60	9.18	13.20	6.06	1.00	.30	.20
" 30.....	6B	14.0	25.50	5.52	18.88	16.64	6.00	5.60	.80
May 7.....	6B	12.4	25.70	3.18	10.80	7.80	7.80	3.40	6.60
" 14.....	6B	15.6	25.90	x	x	x	x	x	x
" 21.....	6B	19.4	25.40	3.00	3.00	4.20	3.00	4.80	3.60
" 28.....	6B	19.0	23.20	6.50	9.00	9.50	3.50	1.10	1.70
Jun. 3.....	6B	18.0	23.90	1.00	15.40	16.00	9.40	.80	.60
" 10.....	6B	20.0	26.80	6.60	11.40	15.00	15.00	7.20	5.40
Totals.....				57.66	124.88	137.04	72.14	64.32	41.32
Averages.....				5.24	11.35	12.45	6.56	5.85	3.75

The great variation in the results is at once apparent. This fluctuation, however, generally occurs in quantitative soil bacteriological work. Samples taken 100 feet apart may often show a variation of 20 to 40 per cent., while those taken from the same depth, only a few feet apart, may vary from 10 to 20 per cent. Commencing at the surface, bacteria increase in number up to the fifth or sixth inch, depending upon the depth of plowing, then rapidly decrease, and disappear at a depth of about seven feet below the surface foot.

Some of the exceptional irregularities in the table can be explained. For instance, on April 23, plot 2A showed twenty-nine million bacteria per cubic centimeter at six inches, thirteen million at eight inches, and eighteen million at ten inches in depth. These high numbers are due to a portion of manure

which had been plowed under the year previous and had not entirely decayed. The piece of manure, to all appearances, had been pressed into some rodent's runway by the horses' hoofs. The average depth of the previous plowing was approximately four inches. The same thing occurred in the samples taken on the same day on plot 4A. The number of bacteria reached forty-two million per cubic centimeter in the fourth inch and thirty-nine million per cubic centimeter in the sixth inch. Again, on April 9, plot 3A showed sixty-one million per cubic centimeter in the sixth. This was also due to manure. Another factor which probably induced these large numbers was the increased temperature, followed by a warm two-inch rain which fell about twenty-four hours before the samples were taken. This view is strengthened by glancing through the twelve tables and comparing estimates for April 23. The sandy soils, which allowed a rapid percolation of water, showed a marked increase in number of bacteria, even in the tenth and twelfth inches, but the more tenacious silt soils showed a decrease in the number of bacteria. The temperature of the silt during the preceding week was 15 degrees C., while on April 23 it had fallen to 14.9 degrees C. The temperature of the sand was 14.9 degrees on April 16, while on April 23 it had risen to 17 degrees C. Plot 4B shows an unaccountable high number in the twelfth inch on the 16th of April.

The tables show that the number of bacteria increased and diminished with more or less regularity. It was also observed on the plate cultures that one species of bacteria would predominate, and at times almost entirely exclude other species. For instance, on April 9 the sixty-one million count consisted almost entirely of minute oval colonies, all of which were apparently of one species.

On May 13 both plots A and B gave results that were exceptional. On the plate cultures there were innumerable tiny colonies so closely set that they could not be accurately estimated. However, estimates placed them at one hundred million per cubic centimeter. One week later, May 20, all the plots showed a marked decrease in the number of organisms. This was perhaps due to the excessive moisture, the ground having been completely saturated for several days; neverthe-

less, the growth on the agar plates was apparently all of one species.

The influence of moisture is perhaps best shown by the results obtained between May 20 and June 2. During this time the soil was more or less saturated. It will be noticed that the number of bacteria diminished quite markedly during this period, and rose again on June 2 nearly to its former height, followed by a decrease on June 9. This was after the per cent. of moisture in the sandy soil had fallen from 27.9 to 25.2 per cent., and from 28.9 to 26.8 in the silt.

A better idea of the influence of deep plowing can be obtained by arranging the averages of tables from 1 to 6 in one table and the averages from tables 7 to 12 in another and comparing the results:

TABLE XIII. Sandy loam.

Depth of sample.	Average number bacteria.					
	1A. Undis- turbed.	2A. Stirred 2 in.	3A. Stirred 4 in.	4A. Stirred 6 in.	5A. Stirred 8 in.	6A. Stirred 10 in.
2 inches.....	14.47	10.82	15.40	9.67	11.04	7.33
4 ".....	11.94	11.62	18.06	15.68	10.68	9.01
6 ".....	7.71	10.84	15.32	14.73	14.09	10.13
8 ".....	5.55	6.74	8.08	7.08	12.64	9.27
10 ".....	5.20	4.66	3.97	3.75	4.19	8.59
12 ".....	2.41	3.31	3.03	2.75	5.47	5.37
Sum of averages.....	47.28	47.99	63.86	53.66	58.11	49.70

TABLE XIV. Silt loam.

Depth of sample.	Average number bacteria.					
	1B. Undis- turbed.	2B. Stirred 2 in.	3B. Stirred 4 in.	4B. Stirred 6 in.	5B. Stirred 8 in.	6B. Stirred 10 in.
2 inches.....	9.85	10.38	11.08	9.82	10.12	5.24
4 ".....	10.34	11.99	10.44	8.91	13.52	11.35
6 ".....	6.81	5.20	6.87	9.69	9.65	12.45
8 ".....	4.33	3.66	4.65	4.51	5.45	6.56
10 ".....	2.58	3.17	4.58	3.04	3.68	5.85
12 ".....	1.45	2.16	3.21	3.26	2.50	3.75
Sum of averages.....	25.36	36.56	40.83	39.23	44.92	45.20

The average in tables XIII and XIV does not give the total number of bacteria in a surface foot of soil, but shows the ratio of the number of bacteria. For instance, in table XIII (sandy soil) the sum of the averages for the unstirred check plot was 47.28 million. The bacterial contents of 3A, 4A and

TABLE XV.

soil, of low humus content, in the most favorable growing area.

If, however, the field were stirred to a depth of ten inches for several years, so as to incorporate humus into the surface ten inches of soil, it is reasonable to suppose that extra deep plowing might be more favorable for the development of bacteria in the sandy soil than it appears to be from the above table. At the same time, however, several years of deep plowing would also be more beneficial to bacterial development in the heavier soils.

These preliminary experiments show that when plowed land is compared with unplowed land it is found that:

Silt—

Plowed 4 inches deep increases the number of bacteria.....	15.46%
Plowed 6 inches deep increases the number of bacteria.....	10.94
Plowed 8 inches deep increases the number of bacteria.....	24.20
Plowed 10 inches deep increases the number of bacteria.....	26.89

Sand—

Plowed 4 inches deep increases the number of bacteria.....	35.06%
Plowed 6 inches deep increases the number of bacteria.....	13.53
Plowed 8 inches deep increases the number of bacteria.....	22.90
Plowed 10 inches deep increases the number of bacteria.....	5.11

Part II.

CONTINUATION OF THE EXPERIMENTS.

On June 20, 1908, the experiments were continued, and determinations were made according to the following plan:

PLOT A (42 x 42 feet).

SUBPLOT A1. Six samples to be taken every 14 days, volumetric method. (Undisturbed.) (See note.)	SUBPLOT A1. Six samples to be taken every 14 days, volumetric method. (Unstirred.)
A2. Six samples to be taken every 14 days, volumetric method. (Stirred 2 inches.)	A2. Six samples to be taken every 14 days, volumetric method. (Stirred 2 inches on March 18 and June 20.)
A3. Six samples to be taken every 14 days, volumetric method. (Stirred 4 inches on March 18.)	A3. Six samples to be taken every 14 days, volumetric method. (Stirred 4 inches on March 18 and June 20.)
A4. Six samples to be taken every 14 days, volumetric method. (Stirred 6 inches on March 18.)	A4. Six samples to be taken every 14 days, volumetric method. (Stirred 6 inches on March 18 and June 20.)
A5. Six samples to be taken every 14 days, volumetric method. (Stirred 8 inches on March 18.)	A5. Six samples to be taken every 14 days, volumetric method. (Stirred 8 inches on March 18 and June 20.)
A6. Six samples to be taken every 14 days, volumetric method. (Stirred 10 inches on March 18.)	A6. Six samples to be taken every 14 days, volumetric method. (Stirred 10 inches on March 18 and June 20.)

NOTE.—Eighteen samples taken by gravimetric method and eighteen samples by volumetric method at the same time and place.

One-half of each subplot in A and also one-half of each subplot in B* were replowed to the same depth as on March 18, in order to see if further stirring of the soil would increase the number and activities of the soil organisms.

The purpose of taking gravimetric samples was to obtain results which would afford a basis for a comparison of the two methods of soil sampling. The gravimetric or weighed samples were secured from the same plots and at the same depth

* Plot B, 42 x 42 feet, duplicate of Plot A.

and place as the volumetric or measured samples. There were two sets of gravimetric samples taken, in order to determine whether drying materially decreased the number of bacteria per gram. One set of gravimetric samples with duplicates was taken immediately to the right of the volumetric samples; the other set with duplicates was taken immediately to the left of the volumetric samples, so as to obtain under the same conditions, as nearly as possible, the same type of soil.

The method employed for taking and caring for individual samples will be discussed under "Quantitative Bacteriological Analysis." After taking the samples to the laboratory, one gravimetric set with duplicates was immediately subjected to culture tests; the other set with duplicates was placed between sheets of sterile filter-paper until air dry. Twenty-four hours was found sufficient for that purpose. They were then treated as were the other samples.

BACTERIAL ACTIVITIES AND INFLUENCING CONDITIONS.

In order to find the influence of aeration upon the biochemic characters of bacteria, determinations were made of the production of ammonia, reduction of nitrates to nitrites, and the production of gas in one-per-cent. solutions of glucose, lactose and saccharose bouillon.

Ammonia Production.—The relative amounts of ammonia in the different samples of soil were determined in the following way: One cubic centimeter of soil was completely suspended in nineteen cubic centimeters of sterile, ammonia-free, distilled water. Two cubic centimeters of this soil suspension were transferred to fifty cubic centimeters of nutrient agar, having a reaction of 1.5 acid to phenolphthalein. The agar was distributed in Erlenmeyer flasks of 250 cc. capacity. The flasks were then closed and allowed to stand for five days. At the end of that time ten cubic centimeters of sterile, nitrogen-free water were introduced and allowed to stand for ten minutes. It was then drawn off and tested for ammonia with Nessler's solution. The color reaction was compared with standard solutions and the results recorded as so many parts of ammonia per million. These standard solutions were prepared by placing fractional percentages of ammonium chloride in nitrogen-free water.

Gas Production.—The production of gas was determined in

the usual way by using the common sugar bouillon media in fermentation tubes, each of which were inoculated with two cubic centimeters of soil suspension.

Reduction of Nitrates to Nitrites.—The denitrifying properties, or reduction of nitrates to nitrites, were determined by inoculating with 2 cubic centimeters of soil suspension, 10 cubic centimeters of Witte's peptone medium, which contained 0.2 gram of potassium nitrate per 1000 cubic centimeters. The tubes were allowed to stand for five days and then tested for nitrites by the addition of sulfanilic acid and naphthylamine chlorid. The results were compared with a standard and recorded as so many parts of nitrites per million.

Temperature.—In the preliminary work the temperature was recorded for the surface foot, but commencing with July 6, the temperature was recorded for each successive second inch up to one foot, or for each soil sample taken.

Acidity of Soil.—At the beginning of the experiment the soil showed acid to litmus at the end of thirty minutes, while at the end of the experiment it showed acid to litmus at the end of ten minutes.

Field Notes.—The condition of the soil surface, the time which had elapsed since the last rain, the amount of precipitation, and other factors such as masses of undecomposed organic matter, plowed soil, unstirred subsoil and burrows or heavy roots were recorded when samples were taken.

Mechanical Analyses.—A mechanical analysis was made of each of the two plots of soil under observation. The results of these analyses were as follows:

SILT SOIL, PLOT B.	
Humus.	3.15 per cent.
Volatiles.	4.86 "
Fine gravel.00 "
Coarse sand.20 "
Medium sand.26 "
Fine sand.46 "
Very fine sand.	24.16 "
Silt.	38.91 "
Clay.	28.00 "
Total.	100.00 per cent.

SANDY SOIL, PLOT A.

Humus.	3.25	per cent.
Volatiles.	5.86	"
Fine gravel.00	"
Coarse sand.28	"
Medium sand.38	"
Fine sand.	3.72	"
Very fine sand.	64.80	"
Silt.	17.18	"
Clay.	4.53	"
Total.	100.00	per cent.

Number and Manner of Taking Samples.—From each half of the subplots which were plowed on March 18, and from each half of the subplots which were plowed on March 18 then re-plowed on June 20, six samples for bacterial determination were taken every fourteenth day. Six samples with duplicates for volumetric bacterial determination were taken from check plot A1 and the same number from check plot B1.

QUANTITATIVE BACTERIOLOGICAL ANALYSIS.

One of the difficult problems in soil bacteriological technique is that relating to the proper method of taking and measuring samples for quantitative determination. It is the general custom of bacteriologists to compare the number of bacteria per gram of dry soil. This is sometimes called the gravimetric or gram method.

In the present experimental work the volumetric method has been used. One cubic centimeter of soil was placed in a sterile test-tube to which was added 19 cc. of sterile distilled water. This was shaken for five minutes, and from each tube, by means of a sterile platinum loop, which was constructed and graduated to hold one cubic millimeter of water, approximately one cubic millimeter of the soil suspension was transferred into 10 cc. of sterile liquefied agar. The inoculated medium was then poured into a sterile Petri dish. This method is undoubtedly inaccurate, as it is difficult to measure such substances as soil in terms of cubic centimeters. However, for the purpose of securing comparative results, as in the present work, either method may be used. In other words, the comparative number of bacteria in soil which has been plowed two, four, six, eight, ten and twelve inches deep may be found by either the volumetric or gravimetric method and expressed as so many bacteria per cubic centimeter or per gram. The final results would appear to be correct so long as one or the other

method of collecting samples is followed with uniformity throughout the work.

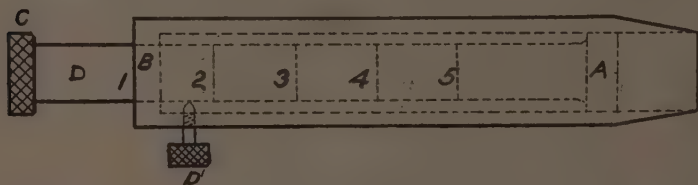


FIG. 1. Soil Sampler.

OBTAINING SAMPLES, AND BACTERIOLOGICAL TECHNIQUE.

The field outfit used in the work consisted of a small steel soil sampler, a spade, a measure, a sample case and a gasoline blow-torch. The soil sampler (fig. 1) is made of steel and consists of a steel tube seven centimeters long with an inside diameter of three-eighths inch and an outside diameter of seven-sixteenths inch. The plunger (D), with a plunger-rod one-eighth inch in diameter, is connected with the cylinder through a one-eighth inch hole (B), and is clamped and held at the desired place by the set-screw (D). The plunger-head (A) is just a working fit inside the cylinder. The plunger-rod (D) is graduated so that the plunger-head allows 1, 2, 3 or 4 cc. of soil to be taken, as desired.

The Sample Case.—The sample case (figs. 2 and 3) is 24 inches long, 18 inches high, and 12 inches wide. A lid, to which is attached the handle, covers the entire top. This is raised and the two catches at either end are released, which allows the case to open. On each side of the case, running the entire length, are three perforated shelves for holding test-tubes. The top shelf is two inches wide and holds 20 test-tubes. The second shelf is four inches wide and holds 20 test-tubes, while the lower shelf is six inches wide and holds the same number of tubes. The entire case holds 120 test-tubes, and when full weighs 7.45 kilograms.

The reason for having the shelves of different widths can be seen in the cut, as it allows the handling of one row of test-tubes without disturbing the others. The test-tube perforations are numbered to correspond with the depth of the sample and number of the plot, so that when a sample is taken the corresponding test-tube can be filled and replaced, thus



FIG. 2. Sample case, closed.



FIG. 3. Sample case, opened.

doing away with needless labeling of tubes. The case can be transferred to the laboratory and the cultures made.

The measure consists of a lath two feet long into which has been bored one-half inch holes every two inches, commencing with the second inch.

Taking the Samples.—To take the samples, dig a hole about a foot square, to the required depth. With a sterile spatula

shave off foreign dirt from one bank. The measure is then placed against this bank and held so that a hole is just two inches below the surface. The sampler is then adjusted to take, say, 1 cc. (see fig. 1). After sterilizing the steel sampler in a blow-torch, with the right hand pass the point through the hole in the measure and force it into the soil, turning it once to free the outside from the soil. With the left hand take the corresponding test-tube from the case. Withdraw the sampler, holding it between the first and second fingers of the right hand, loosen the set-screw with the left, draw the plug (of the test-tube) with the back of the third and fourth fingers of the right hand, flame the mouth of the tube, then stroke the sample with the outer rim of the mouth of the tube, thus obtaining as accurately as possible one cubic centimeter of soil. Insert the sampler about one-half inch within the test-tube, then place the thumb of the right hand upon the plunger-head and force the sample into the test-tube. Withdraw the sampler, replace the plug, and place the tube in the rack.

The samples are then taken to the laboratory and each tube receives 19 cc. of sterilized distilled water, after which it is shaken until all the soil particles have separated.

A millimeter loop of this soil suspension is placed in 10 cc. of liquefied nutrient agar having a reaction of $+1.5$ to phenolphthalein, and the inoculated medium shaken and poured into a Petri dish. The plate cultures were kept at a temperature of about 23 degrees C. The colonies were counted at the expiration of from twenty to twenty-four hours.

TABLE I. Comparative number of bacteria per gram and per cubic centimeter. Plot A, wet.

Depth, inches.	7-6		7-20		8-3	8-17	No. of bacteria.	8-31	No. of bacteria.
	Wt. of sample.	Per cc.	Wt. of sample.	Per cc.	Wt. of sample.	Per gm.	Per cc.	Wt. of sample.	Per cc.
2.....	1.248	10.20	8.17	11.20	8.96	17.60	14.00	1.21	3.60
2D.....	1.25	15.00	12.00	9.60	8.27	1.25	8.00	1.22	2.97
4.....	1.236	22.80	18.40	14.11	10.11	1.23	7.20	1.22	2.14
4D.....	1.30	45.00	34.60	12.00	10.00	1.20	6.40	1.13	5.93
6.....	1.29	11.40	8.83	5.60	4.30	1.24	6.40	1.17	5.00
6D.....	1.40	23.00	11.64	4.30	3.70	1.19	9.60	1.24	4.83
8.....	1.31	8.40	6.41	4.80	4.30	1.20	7.20	1.24	5.32
8D.....	1.23	13.80	11.21	5.60	5.10	1.23	3.60	1.26	4.75
10.....	1.20	6.00	5.00	3.60	2.00	1.19	4.80	1.21	3.47
10D.....	1.19	5.40	4.53	2.40	1.84	1.24	2.00	1.19	1.51
12.....	1.30	6.00	4.65	3.20	2.60	1.30	2.40	1.23	lost
12D.....	1.26	6.00	4.76	4.00	3.33	1.30	2.10	1.22	lost
Totals.....	173.00	130.20	83.60	88.51	77.30	62.18	81.94	66.586	44.60

* D indicates check or duplicate samples.

TABLE II. Number of bacteria per gram. Plot A, dry.

Depth, inches.	7-6		7-20		8-3	8-17	No. of bacteria.	8-31	No. of bacteria.
	Wt. of sample.	Per cc.	Wt. of sample.	Per cc.	Wt. of sample.	Per gm.	Per cc.	Wt. of sample.	Per cc.
2.....	1.04	18.00	17.30	9.60	1.004	no result	13.80	1.036	5.00
2D.....	1.065	34.80	32.60	5.60	1.121	20.40	10.20	1.06	4.80
4.....	1.112	15.60	13.10	7.20	1.085	18.20	10.20	1.06	2.08
4D.....	1.062	32.40	20.50	4.80	1.075	18.00	10.20	1.06	5.67
6.....	1.103	16.80	15.20	4.80	1.05	16.00	10.66	1.06	4.52
6D.....	1.05	15.00	14.28	4.80	1.08	11.20	10.66	1.06	6.90
8.....	1.14	7.20	6.30	3.00	1.07	26.40	2.84	1.021	7.00
8D.....	1.075	13.80	12.80	5.60	1.028	8.60	3.60	1.06	3.88
10.....	1.07	7.10	6.72	3.00	1.069	14.40	1.03	1.04	2.80
10D.....	1.049	1.80	1.70	4.00	1.031	7.20	1.087	1.04	1.84
12.....	1.065	8.40	8.70	3.80	1.025	11.40	1.09	1.04	lost
12D.....	1.078	4.20	3.80	3.80	1.04	3.46	1.20	1.09	1.29
Totals.....	175.20	163.00	55.20	51.60	141.80	130.19	79.60	2.65	lost

39.48

TABLE III. Number of bacteria per gram. Plot B, wet.

Depth, inches.	7-9		7-23		No. of bacteria.		8-6		No. of bacteria.		8-20		No. of bacteria.		9-3		No. of bacteria.	
	Wt. of sample.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.
2.....	1.135	19.20	16.00	14.80	14.21	1.21	1.21	3.00	2.48	1.11	1.11	2.00	1.80	1.12	2.40	2.14	2.40	2.14
2D.....	1.129	19.20	16.30	12.60	11.00	1.20	1.20	10.20	8.50	1.11	1.11	12.80	11.50	1.16	7.80	6.61	7.80	6.61
4.....	1.20	7.20	6.00	2.40	2.11	1.24	1.24	2.40	1.90	1.12	1.12	4.80	4.28	1.22	5.40	4.50	5.40	4.50
4D.....	1.134	36.80	32.40	12.60	10.99	1.19	1.19	5.40	4.50	1.16	1.16	7.80	6.20	1.22	4.80	3.90	4.80	3.90
6.....	1.12	3.00	2.67	2.40	2.01	1.10	1.10	4.20	3.818	1.20	1.20	5.2	4.33	1.33	4.80	3.60	4.80	3.60
6D.....	1.021	9.60	9.40	2.40	2.14	1.20	1.20	1.80	1.80	1.09	1.09	7.20	6.60	1.01	10.80	10.60	10.80	10.60
8.....	1.032	5.56	5.42	1.21	3.47	1.14	1.14	1.20	1.05	1.10	1.10	4.40	3.909	1.19	4.80	4.03	4.80	4.03
8D.....	1.23	3.20	2.60	1.80	1.52	1.20	1.20	3.00	2.50	1.00	1.00	4.40	4.40	1.07	7.20	6.72	7.20	6.72
10.....	1.228	2.40	1.54	1.20	1.165	1.14	1.14	.06	.05	1.20	1.20	2.20	1.80	1.11	4.80	4.32	4.80	4.32
10D.....	1.129	1.60	1.41	.82	.65	1.16	1.16	.15	.13	1.14	1.14	4.40	3.80	1.13	7.80	6.90	7.80	6.90
12.....	1.134	1.02	.0175	1.19	.084	1.20	1.20	.04	.04	1.15	1.15	2.80	.37	1.12	2.40	2.10	2.40	2.10
12D.....	1.232	2.40	1.93	1.13	.141	1.10	1.10	.10	.0909	1.16	1.16	2.80	2.40	1.21	3.80	3.40	3.80	3.40
Totals.....	105.18	91.41	54.48	48.401	31.56	26.34	46.86	42.85	66.80	58.82	66.80	58.82

TABLE IV. Number of bacteria per gram. Plot B, dry.

Depth, inches.	7-9		7-23		No. of bacteria.		8-6		No. of bacteria.		8-20		No. of bacteria.		9-3		No. of bacteria.	
	Wt. of sample.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.	Wt. of sample.	Per cc.	Per gm.	Per cc.
2.....	1.035	9.60	9.20	6.00	5.76	1.036	1.036	10.20	9.80	1.02	1.02	6.00	5.88	1.03	4.00	3.78	4.00	3.78
2D.....	1.024	80.40	29.50	9.60	9.20	1.035	1.035	1.60	1.035	1.0319	1.0319	9.00	8.80	1.024	4.20	4.10	4.20	4.10
4.....	1.01	6.40	6.80	1.036	5.70	1.029	1.029	3.60	3.40	1.04	1.04	5.40	5.19	1.018	4.60	4.50	4.60	4.50
4D.....	1.034	8.00	7.70	1.035	6.00	5.70	1.03	11.40	11.06	1.026	1.026	4.80	4.60	1.043	4.20	4.00	4.20	4.00
6.....	1.02	4.00	4.70	.88	.36	1.021	1.021	4.20	4.10	1.034	1.034	3.60	3.40	1.034	3.40	3.20	3.40	3.20
6D.....	1.021	4.80	3.90	1.03	2.33	1.021	1.021	3.60	3.50	1.04	1.04	6.60	6.34	1.021	9.00	8.80	9.00	8.80
8.....	1.032	8.00	7.90	.26	.25	1.019	1.019	1.20	1.10	1.036	1.036	8.40	8.10	1.021	3.00	2.90	3.00	2.90
8D.....	1.03	5.60	5.43	.28	.27	1.021	1.021	4.20	4.10	1.03	1.03	6.00	5.82	1.04	3.00	2.83	3.00	2.83
10.....	1.028	2.40	2.30	1.033	.20	.19	1.034	1.20	1.10	1.028	1.028	6.00	5.80	1.06	3.40	3.20	3.40	3.20
10D.....	1.029	2.40	2.30	.86	.85	1.021	1.021	.12	.11	1.03	1.03	3.40	3.30	1.051	lost	lost	lost	lost
12.....	1.032	4.60	4.60	1.03	2.35	1.04	1.04	.44	.42	1.024	1.024	4.20	4.10	1.04	4.20	4.03	4.20	4.03
12D.....	1.034	1.60	1.50	.28	.26	1.029	1.029	.20	.19	1.029	1.029	.46	.44	1.051	lost	lost	lost	lost
Totals.....	88.00	85.33	34.16	32.72	41.96	39.91	63.86	61.77	43.00	41.39	43.00	41.39

TABLE V—Plot A (sandy loam). Plowed March 13. Number of bacteria per cubic centimeter, per cent. of moisture, depth in inches, and temperature.

DATE.	Second inch.			Fourth inch.			Sixth inch.			Eighth inch.			Tenth inch.			Twelfth inch.			Plot.	Treatment.
	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.		
Jul. 6.....	22.0	11.4	20.0	19.6	11.4	21.2	18.9	9.0	22.4	18.1	10.80	23.0	18.0	2.40	23.0	18.0	4.20	24.0	A1	Undisturbed.
Aug. 3.....	20.5	6.4	24.8	21.0	12.0	24.3	20.9	9.6	26.0	20.8	14.40	28.3	20.4	6.00	25.8	19.9	3.60	28.1		
Aug. 17.....	26.3	8.4	21.0	24.0	22.7	23.0	22.7	13.2	20.0	22.7	3.00	21.0	22.5	1.16	21.0	22.4	1.14	22.4		
.. 31.....	26.2	6.6	20.0	26.2	15.6	23.0	25.8	11.4	24.0	24.7	10.20	23.0	24.3	2.40	24.0	24.2	1.80	25.0		Plowed 2 inches.
Jul. 6.....	22.0	14.4	21.5	19.6	13.0	22.0	18.0	24.0	25.0	18.1	19.20	25.5	18.0	9.00	25.7	18.0	3.00	26.8	A2	
Aug. 3.....	20.8	21.6	24.8	21.0	21.6	23.0	20.9	18.0	23.0	20.8	18.00	26.0	20.4	4.80	26.1	19.9	1.20	25.8		
Aug. 17.....	26.3	13.2	26.0	26.2	11.4	24.0	26.8	10.2	24.0	24.7	6.00	22.0	22.5	3.60	22.9	22.4	2.26	24.0		Plowed 4 inches.
.. 31.....	26.2	8.4	26.0	26.2	6.6	21.0	25.3	5.4	22.0	25.6	9.60	26.0	24.3	3.40	26.0	24.2	2.40	28.0		
Jul. 6.....	22.0	15.0	18.9	19.6	16.8	21.1	18.9	19.2	22.5	18.0	11.40	23.9	18.0	9.60	24.8	18.0	9.00	25.0	A3	
Aug. 3.....	20.5	16.0	26.3	21.0	16.6	25.6	20.9	23.4	25.7	20.8	12.00	26.2	20.4	6.00	25.5	19.9	8.40	25.2		Plowed 6 inches.
Aug. 17.....	26.3	6.6	26.0	26.2	12.8	23.0	25.8	6.0	23.0	22.7	4.80	24.0	22.5	4.80	24.0	22.0	1.80	26.0		
.. 31.....	21.3	11.4	20.0	26.2	10.8	23.0	25.3	3.6	24.0	24.7	3.60	25.2	24.3	6.40	25.0	24.2	.88	25.2		
Jul. 6.....	22.0	12.6	20.0	19.6	27.0	20.0	18.9	21.0	21.1	18.1	20.40	22.8	18.0	23.80	22.8	18.0	5.40	26.6	A4	Plowed 8 inches.
Aug. 3.....	20.5	13.2	23.7	21.3	16.8	24.5	20.9	25.4	25.1	20.4	12.00	26.7	20.4	10.80	26.2	19.9	6.00	26.0		
Aug. 17.....	26.3	9.0	23.3	26.2	6.0	23.0	22.7	3.6	23.9	22.7	4.80	23.8	22.5	3.60	21.0	22.4	1.66	24.7		
.. 31.....	26.2	4.8	18.0	26.2	10.8	22.0	25.3	9.0	22.0	25.6	9.40	25.4	24.3	8.00	26.0	24.2	1.20	26.3		Plowed 10 inches.
Jul. 6.....	22.0	27.0	20.6	19.6	38.4	20.8	18.9	32.4	21.4	18.1	9.96	21.2	18.0	40.07	23.4	18.0	11.40	24.5	A5	
Aug. 3.....	20.5	27.6	23.3	21.0	30.0	25.8	20.9	16.8	25.2	20.8	22.80	26.4	20.4	12.00	26.7	19.9	8.40	27.0		
Aug. 17.....	26.3	4.8	19.0	26.3	4.8	24.0	28.7	7.2	24.0	22.7	15.60	25.5	22.5	3.60	25.1	22.4	9.60	26.1		Plowed 10 inches.
.. 31.....	26.2	4.2	20.0	25.2	7.2	20.0	25.8	4.2	25.0	24.5	12.00	25.4	22.5	4.80	15.0	25.2	6.60	26.0		
Jul. 6.....	22.0	50.4	19.0	19.6	48.0	20.2	18.9	30.0	21.2	18.1	2.22	22.5	18.0	18.00	23.4	18.0	24.50	25.8	A6	
Aug. 3.....	20.5	20.4	21.2	21.0	13.2	23.0	20.9	27.6	23.6	20.4	10.80	23.0	20.4	4.80	24.3	19.9	6.00	24.6		Plowed 10 inches.
Aug. 17.....	26.3	9.0	20.0	26.3	7.2	20.1	22.7	10.8	23.0	22.7	8.40	23.4	22.5	7.20	24.0	22.4	3.00	24.1		
.. 31.....	26.2	8.0	20.0	26.2	3.6	21.0	25.8	3.6	26.0	24.5	8.00	23.0	24.3	6.60	23.9	24.2	.82	26.0		

x = spreading growth.

TABLE VI.—PLOT A (sandy loam). Plowed March 18 and June 20. Number of bacteria per cubic centimeter, per cent. of moisture, depth in inches, and temperature.

DATE.	Second inch.			Fourth inch.			Sixth inch.			Eighth inch.			Tenth inch.			Twelfth inch.			Plot.	Treatment.
	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.		
Jul. 6.....	22.0	24.6	20.0	19.6	19.8	21.2	18.9	36.6	22.4	18.1	20.2	23.0	18.0	9.60	23.00	18.0	3.60	23.0	A1	Undisturbed.
Aug. 20.....	20.5	25.2	24.8	21.0	20.4	24.3	20.9	19.2	26.0	20.8	14.4	28.3	20.4	10.80	25.80	19.9	74	23.5		
Aug. 3.....	25.6	16.8	21.3	24.3	13.2	20.0	22.7	7.2	21.0	22.7	9.6	21.0	22.5	2.40	21.00	23.4	.74	23.0		
Aug. 17.....	26.3	9.0	20.0	26.2	15.6	23.0	25.8	11.4	24.0	24.7	10.2	23.0	24.3	2.40	24.00	24.2	1.80	23.0		
Aug. 31.....	26.2	6.6	24.0	25.2	5.4	21.0	25.6	5.4	21.0	25.6	6.0	21.0	25.7	2.40	24.00	25.7	1.80	23.0	A2	Plowed 2 inches.
Jul. 6.....	22.0	27.6	21.6	19.6	16.2	22.0	18.9	18.6	25.0	18.1	18.6	25.5	18.0	15.60	25.70	18.0	5.40	26.8		
Aug. 20.....	20.5	21.8	24.8	21.0	24.0	23.0	20.9	12.0	28.0	20.8	6.0	26.0	20.4	3.60	26.10	19.9	3.60	25.8		
Aug. 3.....	25.6	9.6	20.0	24.3	8.4	20.0	22.7	12.0	20.0	22.7	15.6	22.0	22.5	3.60	22.00	22.4	1.60	24.0		
Aug. 17.....	26.3	13.2	25.0	26.2	11.4	24.0	25.8	10.2	24.0	24.7	5.4	26.0	24.3	3.40	26.00	24.2	2.40	23.0		
Aug. 31.....	26.2	8.4	18.0	25.2	6.6	21.0	25.3	5.4	22.0	25.6	9.6	25.0	25.7	3.00	24.00	25.7	.26	26.0	A3	Plowed 4 inches.
Jul. 6.....	22.0	22.8	18.8	19.6	19.8	21.0	18.9	13.2	22.5	18.1	16.2	23.0	18.0	7.80	24.80	18.0	1.80	25.1		
Aug. 20.....	20.5	12.0	26.3	21.0	32.4	26.6	20.9	13.2	25.7	20.8	8.4	26.2	20.4	6.00	25.50	19.9	2.40	25.2		
Aug. 3.....	25.6	6.0	21.0	24.8	8.4	23.4	22.7	7.2	24.9	22.7	5.4	25.0	22.5	1.20	26.00	22.4	2.40	24.9		
Aug. 17.....	26.3	6.0	20.0	26.2	4.8	23.0	21.8	10.2	24.0	24.7	8.0	24.3	24.3	.46	25.90	21.7	.66	26.0		
Aug. 31.....	26.7	6.6	23.0	25.2	3.0	19.0	25.8	8.6	23.0	25.6	4.0	23.4	25.7	.30	26.00	21.7	.30	25.4	A4	Plowed 6 inches.
Jul. 6.....	22.0	15.0	22.3	19.6	15.6	22.0	18.9	18.9	21.6	18.1	16.2	26.5	18.0	5.40	25.70	18.0	4.80	27.0		
Aug. 20.....	20.5	20.4	27.5	21.0	27.6	27.0	20.9	46.8	26.5	20.8	16.2	25.8	20.4	8.40	25.40	19.9	4.80	25.4		
Aug. 3.....	25.6	11.4	21.0	24.8	9.6	22.0	22.7	13.2	22.9	22.7	12.6	23.0	22.5	4.46	23.70	22.4	2.40	25.0		
Aug. 17.....	26.3	15.2	24.0	26.5	6.6	24.5	25.8	12.6	24.0	24.7	7.8	25.9	24.3	4.20	26.30	24.2	3.00	26.0		
Aug. 31.....	26.2	6.1	17.0	25.2	6.1	22.0	25.3	8.4	23.4	25.6	3.0	25.2	25.7	8.00	25.00	21.7	5.00	27.0	A5	Plowed 8 inches.
Jul. 6.....	22.0	46.2	19.2	19.6	24.6	22.2	18.9	15.6	24.0	18.1	19.2	24.9	18.0	10.80	26.10	18.0	4.80	26.0		
Aug. 20.....	20.5	15.6	25.7	21.0	14.4	24.6	20.9	28.4	26.0	20.8	31.2	25.9	20.4	12.00	26.10	19.9	1.20	27.3		
Aug. 3.....	25.6	3.6	19.0	24.3	23.4	26.7	22.7	7.8	25.8	22.7	10.8	26.0	22.5	15.00	27.04	22.4	6.00	27.1		
Aug. 17.....	26.3	12.0	20.0	26.5	3.6	21.0	25.8	9.6	22.1	24.7	6.0	25.0	24.5	6.60	26.20	24.2	4.20	26.0		
Aug. 31.....	26.2	8.4	24.3	25.2	4.9	23.0	25.8	6.0	22.1	25.6	7.8	26.1	25.7	4.80	24.80	21.7	1.80	23.2	A6	Plowed 10 inches.
Jul. 6.....	22.0	19.2	19.6	19.6	17.4	22.1	18.9	9.6	22.9	18.1	16.8	23.7	18.0	7.20	23.10	11.8	8.40	24.2		
Aug. 20.....	20.5	12.0	24.2	21.0	14.4	24.6	20.9	19.2	24.9	20.8	4.2	26.9	20.4	13.20	20.20	19.9	8.40	26.5		
Aug. 3.....	25.6	4.2	19.0	24.8	10.8	21.0	22.7	8.4	24.0	22.7	5.4	23.0	22.5	6.00	24.00	22.3	6.00	25.0		
Aug. 17.....	26.3	4.8	20.0	26.5	10.8	21.0	25.8	8.4	22.0	24.7	4.8	24.0	24.3	9.60	24.30	24.2	4.80	25.2		
Aug. 31.....	26.2	7.2	20.0	25.2	7.2	21.0	25.3	6.0	21.5	25.6	6.0	25.0	25.7	5.40	24.00	21.7	4.20	25.2		

TABLE VII—Plot B (silt loam). Plowed March 13. Number of bacteria per cubic centimeter, per cent. of moisture, depth in inches, and temperature.

DATE.	Second inch.			Fourth inch.			Sixth inch.			Eighth inch.			Tenth inch.			Twelfth inch.			Plot.	Treatment.
	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.		
Jul. 9.	25.4	25.20	17.0	23.1	36.0	17.9	23.9	8.40	19.0	22.3	2.40	20.0	21.9	2.40	20.0	21.0	7.20	23.0	B1	Undisturbed.
Jul. 23.	28.0	4.80	18.0	26.0	13.2	18.4	24.9	.30	19.0	26.8	2.40	19.9	25.0	.10	26.0	24.7	.18	25.0		
Aug. 6.	28.6	2.40	20.0	27.3	1.8	20.9	26.7	.40	24.0	26.8	.24	26.9	24.0	.02	26.9	26.6	.02	26.0		
Aug. 20.	21.3	7.20	26.0	20.2	4.8	25.4	19.8	6.00	26.8	19.7	3.00	27.0	20.3	6.00	27.2	20.3	1.80	27.0		
Sep. 3.	22.0	10.20	26.0	20.0	5.4	27.0	20.7	15.60	27.0	20.9	4.20	29.0	21.8	2.40	20.9	22.0	1.80	23.0		Plowed 2 inches.
Jul. 9.	25.4	18.00	25.0	23.1	40.8	25.0	23.9	19.20	27.0	22.3	4.80	27.4	21.9	4.80	27.8	21.0	2.80	26.9	B2	
Jul. 23.	28.0	.84	10.0	26.0	4.8	24.6	24.9	4.80	13.0	24.9	.12	16.0	25.0	.08	17.0	24.7	.08	11.0		
Aug. 6.	28.6	.38	15.0	27.3	7.7	18.0	26.7	.14	20.0	26.8	.18	23.0	26.9	.08	20.0	26.6	.02	21.0		
Aug. 20.	21.3	6.00	23.0	20.2	6.6	lost	19.8	5.40	20.0	19.7	.26	20.0	20.3	.38	19.0	20.3	.38	26.0		
Sep. 3.	22.0	4.80	18.0	20.0	7.2	21.0	20.7	5.40	lost	20.9	6.00	20.0	21.8	4.80	22.0	22.0	.18	16.0		Plowed 4 inches.
Jul. 9.	25.4	19.20	18.0	23.1	15.6	20.0	22.9	7.20	19.0	22.3	7.20	17.0	21.9	4.80	20.0	21.0	3.60	26.0	B3	
Jul. 23.	28.0	6.00	15.4	26.0	10.0	17.0	24.9	.04	17.0	24.9	.06	11.0	25.0	.00	11.0	24.7	.01	29.0		
Aug. 6.	28.6	3.60	18.0	27.3	4.2	17.0	26.7	.28	20.0	26.8	.68	21.0	26.9	.58	21.0	26.6	.08	28.0		
Aug. 20.	21.3	14.40	23.0	20.2	16.8	17.6	19.8	10.20	19.0	19.7	.16	22.0	20.3	1.12	23.0	20.3	.21	21.0		
Sep. 3.	22.0	7.20	18.0	20.0	6.0	20.0	20.7	10.80	19.0	20.9	3.60	17.0	21.8	3.60	20.0	22.0	.12	26.0		Plowed 6 inches.
Jul. 9.	25.4	3.60	16.0	23.1	28.8	20.0	22.9	24.00	17.0	22.3	3.60	21.0	21.9	4.20	18.0	21.0	1.20	22.0	B4	
Jul. 23.	28.0	6.00	17.0	26.0	6.0	17.0	24.9	3.60	12.0	24.9	.16	15.0	25.0	.08	15.0	24.7	.03	11.0		
Aug. 6.	28.6	.18	18.0	27.3	1.8	19.0	26.7	4.20	20.0	26.8	.02	18.0	26.9	.24	20.0	26.6	.08	23.0		
Aug. 20.	21.3	5.40	21.0	20.2	11.4	27.0	19.8	4.80	22.0	19.7	.36	23.0	20.3	.18	18.0	20.3	.62	23.0		
Sep. 3.	22.0	5.40	16.0	20.0	10.8	20.0	20.7	8.40	17.0	20.9	4.80	21.0	21.8	3.60	18.0	22.0	3.60	22.0		Plowed 8 inches.
Jul. 9.	25.4	30.00	16.0	23.1	30.0	17.0	22.9	4.20	22.0	22.3	8.40	20.7	21.9	6.00	19.0	21.0	4.80	22.0	B5	
Jul. 23.	28.0	.04	13.0	26.0	4.8	13.0	24.9	48.00	18.2	24.9	3.60	10.0	25.0	.06	10.0	24.7	.04	12.0		
Aug. 6.	28.6	2.40	19.0	27.3	2.4	18.0	26.7	3.00	19.0	26.8	.16	19.0	26.9	.24	17.0	26.6	1.80	21.0		
Aug. 20.	21.3	6.00	20.0	20.2	30.6	22.0	19.8	10.20	23.0	19.7	8.00	23.0	20.3	2.22	24.0	20.3	.34	23.0		
Sep. 3.	22.0	7.80	16.0	20.0	9.0	17.0	20.7	4.80	20.0	20.9	8.00	20.7	21.8	3.00	19.0	22.0	3.00	22.0		Plowed 10 inches.
Jul. 9.	25.4	26.40	19.0	23.1	33.6	20.0	22.9	34.80	20.0	22.3	27.60	20.0	21.9	7.20	20.0	21.0	4.80	22.0	B6	
Jul. 23.	28.0	1.80	19.0	26.0	2.4	13.0	24.9	3.60	15.0	24.9	.32	18.0	25.0	.10	19.4	24.7	.12	20.4		
Aug. 6.	28.6	1.80	19.0	27.3	6.0	20.0	26.7	1.00	18.0	26.8	.12	19.0	26.9	.06	23.0	26.6	.44	21.0		
Aug. 20.	21.3	6.60	20.2	20.8	10.7	17.0	19.8	4.20	17.0	19.7	4.80	20.8	20.3	3.00	23.0	20.3	1.80	24.0		
Sep. 3.	22.0	4.80	19.0	20.0	7.3	20.0	20.7	4.20	20.0	22.9	5.40	20.0	21.8	4.20	20.0	22.0	2.40	22.0		

TABLE VIII.—Plot B (silt loam). Plowed March 13 and June 20. Number of bacteria per cubic centimeter, per cent. of moisture, depth in inches, and temperature.

DATE.	Second inch.			Fourth inch.			Sixth inch.			Eighth inch.			Tenth inch.			Twelfth inch.			Plot.	Treatment.
	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.	T.	B.	W.		
Jul. 9..	25.4	31.2	17.0	23.1	38.40	17.0	22.9	13.20	17.0	22.3	8.40	19.0	21.9	9.60	20.0	21.0	4.80	23.0	B1	Undisturbed.
Jul. 23..	23.0	6.0	12.0	26.0	7.20	18.4	24.8	.30	19.0	24.9	3.60	18.0	25.0	3.60	23.0	24.7	.10	25.0		
Aug. 6..	23.6	2.4	15.0	27.3	1.80	19.0	26.7	6.00	24.0	25.8	.24	21.0	26.9	6.02	20.0	26.6	.02	24.0		
Aug. 20..	21.3	7.2	28.0	20.2	4.80	21.0	19.8	5.40	17.0	19.7	3.00	20.0	20.3	6.00	20.3	20.3	1.80	25.0	B2	Plowed 2 inches.
Sep. 3..	22.0	10.2	11.0	20.0	5.40	17.0	20.7	15.60	17.0	20.9	4.20	19.0	21.8	2.40	20.0	22.0	1.80	23.0		
Jul. 9..	25.4	16.8	18.0	23.1	26.46	15.1	22.0	19.20	15.2	22.3	4.80	20.0	21.9	2.40	22.0	21.0	2.40	16.0		
Jul. 23..	23.0	13.2	10.0	26.9	7.20	24.6	24.9	2.00	13.0	24.9	3.80	16.0	25.0	.12	17.0	24.7	.16	11.0	B3	Plowed 4 inches.
Aug. 6..	23.6	3.8	15.0	27.3	7.00	18.0	26.7	.14	20.0	26.8	1.80	23.0	26.9	.08	20.0	26.6	.08	21.0		
Aug. 20..	21.3	6.0	23.0	20.0	6.60	19.0	19.8	5.40	20.0	19.7	.26	20.0	20.3	.38	19.0	20.3	.38	16.0		
Sep. 3..	22.0	4.8	18.0	20.7	7.20	15.1	20.7	5.40	lost	20.9	6.00	20.0	21.8	.48	22.0	22.0	1.80	20.0	B4	Plowed 6 inches.
Jul. 9..	25.4	26.4	17.0	23.1	30.00	20.0	22.9	7.20	21.0	22.3	6.00	20.0	21.9	3.60	23.0	21.0	.60	23.0		
Jul. 23..	23.0	6.0	13.6	26.0	9.60	13.7	24.9	.16	23.0	24.9	12.00	13.8	25.0	.36	12.0	24.7	.02	18.0		
Aug. 6..	23.6	13.2	19.0	27.3	.28	14.0	26.7	.30	18.0	26.8	5.80	26.0	26.9	.20	19.0	26.6	.72	22.0	B5	Plowed 8 inches.
Aug. 20..	21.3	10.2	18.0	20.2	18.00	20.0	19.7	14.40	23.0	19.7	4.80	23.0	20.3	3.60	23.0	20.3	.30	23.0		
Sep. 3..	22.0	7.2	18.0	20.0	10.80	17.0	20.7	4.20	21.0	20.9	5.40	23.0	21.8	3.60	23.0	22.0	4.80	23.0		
Jul. 9..	25.4	39.6	15.0	23.1	40.80	18.0	22.9	7.20	20.0	22.3	4.80	20.0	21.9	2.40	19.0	21.0	.60	20.0	B6	Plowed 10 inches.
Jul. 23..	23.0	3.6	16.0	26.0	19.20	25.0	24.9	.08	15.0	24.9	2.40	19.0	25.0	.30	12.0	24.7	.30	14.2		
Aug. 6..	23.6	7.2	16.0	27.3	6.00	20.0	26.7	.96	23.0	26.8	4.80	18.0	26.9	.04	22.0	26.6	.12	21.0		
Aug. 20..	21.3	13.2	21.0	20.2	17.40	25.0	19.8	11.40	21.0	19.7	4.20	16.0	20.3	1.80	23.4	20.3	1.40	24.0	B5	Plowed 8 inches.
Sep. 3..	22.0	7.2	19.0	20.0	12.00	21.0	20.7	4.80	lost	20.9	2.40	25.0	21.8	3.60	22.9	22.0	3.00	23.0		
Jul. 9..	25.4	16.8	18.0	23.1	27.60	17.0	22.9	10.80	21.0	22.3	7.20	23.0	21.9	1.20	23.0	21.0	2.40	23.0		
Jul. 23..	23.0	6.0	24.0	26.0	8.40	22.0	24.9	7.20	18.0	24.9	3.60	16.0	25.0	.14	18.0	24.7	.02	23.0	B6	Plowed 10 inches.
Aug. 6..	23.6	4.8	20.0	27.3	3.60	23.0	26.7	18.00	23.0	26.8	.32	15.0	26.9	.04	21.0	26.6	.08	21.0		
Aug. 20..	21.3	3.6	23.0	20.2	18.60	24.0	19.8	11.40	23.0	19.7	.12	27.0	20.3	.30	27.0	20.3	1.20	25.0		
Sep. 3..	22.0	4.8	17.0	20.0	5.40	20.0	20.7	6.60	21.0	20.9	9.00	20.0	21.8	10.20	24.0	22.0	7.20	23.0	B6	Plowed 10 inches.
Jul. 9..	25.4	21.6	19.0	23.1	33.60	21.0	22.9	15.60	20.0	22.3	7.20	25.0	21.9	7.00	22.0	21.0	3.60	23.0		
Jul. 23..	23.0	6.0	24.0	26.0	4.80	18.0	24.9	3.60	15.0	24.9	.70	10.0	25.0	.04	21.0	24.7	.04	12.0		
Aug. 6..	23.6	3.6	15.0	27.7	4.80	18.0	26.7	3.60	18.0	26.8	7.20	18.0	26.9	5.40	20.0	26.6	1.20	19.0	B6	Plowed 10 inches.
Aug. 20..	21.3	5.4	23.0	20.2	9.60	23.0	19.8	10.20	23.0	19.7	14.40	23.0	20.3	8.40	25.0	20.3	2.40	26.0		
Sep. 3..	22.0	7.8	16.0	20.0	7.20	18.0	20.7	9.60	20.0	20.9	8.40	20.0	21.8	4.80	19.0	22.0	.30	20.0		

A COMPARISON OF THE GRAVIMETRIC AND VOLUMETRIC METHODS.

Tables I to IV give the comparative results obtained by gravimetric and volumetric methods. The samples were taken by means of the sampler described above. One cubic centimeter of soil was taken each time, placed in a sterile test-tube and weighed on an analytic balance. In every case duplicate samples were obtained and analyzed. In the following tables the weight of each sample is given in the first column, the number of bacteria per cubic centimeter is given in the second, and the number of bacteria per gram is given in the third column.

A glance at tables I to IV will show a variation in weights of samples varying in table I from 1.19 to 1.4 grams per cubic centimeter, from 0.965 to 1.14 grams in table II, from 1.021 to 1.232 grams in table III, and from 1.01 grams to 1.035 grams in table IV. The comparison also shows remarkable differences in weight between many samples and their duplicates, which were taken side by side. For instance, in table IV the fourth inch gives 1.01 grams while the duplicate gives 1.034. In the sandy soil (table II) the difference becomes more marked, showing in the twelfth inch a difference of 0.113 of a gram.

In tables I to IV the number of bacteria per cubic centimeter and per gram shows, in duplicate samples of soil, variations which are somewhat proportionate to the variations in weight of samples. These variations in quantitative findings represent the inaccuracy which is due to the volumetric method of taking soil samples. It may be assumed that such variations or inaccuracies will always occur from soil samples which are measured in terms of cubic centimeters. However, in general comparative soil bacteriological work these variations in number of bacteria, due to the volumetric method of procedure, may not be greater than those variations which might occur as a result of differences in specific gravities of different samples of soil, should the soil samples be taken by the gravimetric method. For instance, two types of soil, Marshall silt loam and sandy loam, were used in this work. The volume weight of the silt loam soil is 1.11 and the volume weight of the sandy loam is 1.35. Their weights stand in the ratio of 1:1.21; that is, an acre-foot of the sandy loam weighs 1.21 times as much as an acre-foot of the silt loam. Therefore,

should samples be collected from the above types of soil by the gravimetric method and the comparative results expressed as so many bacteria per gram, the number of bacteria in an acre-foot of the sandy loam would in reality be compared with the number of bacteria in 1.21 acre-feet of the silt loam.

In quantitative bacteriological work, when comparative results are desired, the volumetric method has the following possible advantages over the gravimetric method:

1. It is more convenient.
2. It allows less chance for contamination.
3. It represents more accurately an aliquot part of an acre-foot of soil.

The last advantage appears to be true because of the difference in specific gravity of soils.

Drying Effect.—When we compare tables I and II with III and IV, we find no appreciable difference in results between those soil samples which were allowed to dry twenty-four hours before inoculation and those from which inoculations were made immediately after collection.

TABLE IX—PLOT A. Summarized total number bacteria given in table V.

DEPTH.	A1. Undis- turbed.	A2. Plowed 2 in.	A3. Plowed 4 in.	A4. Plowed 6 in.	A5. Plowed 8 in.	A6. Plowed 10 in.
2 inches.....	43.80	72.00	53.40	44.40	72.00	98.00
4 ".....	58.80	57.40	43.20	68.40	80.40	75.50
6 ".....	42.60	70.80	60.00	66.60	69.00	85.50
8 ".....	46.40	58.20	36.60	52.20	63.96	47.30
10 ".....	13.36	20.80	28.20	45.60	64.07	43.10
12 ".....	11.54	7.12	20.48	15.46	38.50	34.12
Totals.....	216.50	286.32	241.68	292.66	393.93	383.52

TABLE X—PLOT A. Summarized total number of bacteria given in table VI.

DEPTH.	A1. Undis- turbed.	A2. Plowed 2 in.	A3. Plowed 4 in.	A4. Plowed 6 in.	A5. Plowed 8 in.	A6. Plowed 10 in.
2 inches.....	82.20	80.60	58.40	66.10	85.80	47.40
4 ".....	74.40	66.60	68.40	65.50	70.90	54.60
6 ".....	79.80	58.20	47.40	102.60	65.40	51.00
8 ".....	60.40	55.20	37.00	39.60	75.00	37.20
10 ".....	27.60	29.20	15.76	21.46	49.20	41.40
12 ".....	8.54	13.26	7.56	20.00	18.00	31.80
Totals.....	392.94	303.06	229.52	315.26	354.30	263.40

TABLE XI—PLOT B. Summarized total number bacteria given in table VII.

DEPTH.	B1. Undis- turbed.	B2. Plowed 2 in.	B3. Plowed 4 in.	B4. Plowed 6 in.	B5. Plowed 8 in.	B6. Plowed 10 in.
2 inches.....	49.80	30.02	50.40	20.58	46.24	42.00
4 ".....	61.20	60.10	52.60	53.80	76.80	60.00
6 ".....	30.70	34.94	18.92	45.00	70.20	47.80
8 ".....	12.24	11.36	11.70	8.94	18.16	38.24
10 ".....	10.92	13.06	10.10	3.30	13.68	14.56
12 ".....	11.00	3.46	4.02	5.51	9.98	9.56
Totals.....	175.86	152.94	147.74	147.13	235.06	212.16

TABLE XII—PLOT B. Summarized total number bacteria given in table VIII.

DEPTH.	B1. Undis- turbed.	B2. Plowed 2 in.	B3. Plowed 4 in.	B4. Plowed 6 in.	B5. Plowed 8 in.	B6. Plowed 10 in.
2 inches.....	57.00	44.60	63.00	70.80	42.00	44.40
4 ".....	57.60	54.40	68.68	95.40	63.60	60.00
6 ".....	35.50	32.14	26.26	24.44	54.00	42.60
8 ".....	19.44	13.16	28.78	14.20	20.24	37.24
10 ".....	21.62	3.46	11.36	7.92	11.88	25.64
12 ".....	8.52	4.82	6.44	5.42	10.90	7.54
Totals.....	199.68	152.58	204.52	218.18	202.62	217.42

TABLE XIII.

DATE.	Plot.	Am- monia, parts per million.	Nitrites, parts per million.	Gas production.		
				Glucose bouillon, in cc.	Lactose bouillon, in cc.	Saccha- rose bouillon, in cc.
Jul. 20.....	A1	25	75	0.00	0.00	0.0
" 20.....	A2	50	75	7.70	1.25	0.0
" 20.....	A3	50	75	4.50	1.75	0.9
" 20.....	A4	1	40	5.80	0.00	0.0
" 20.....	A5	75	1	5.80	0.00	0.0
" 20.....	A6	75	14	5.50	0.00	0.0
Totals.....		276	280			
Aug. 3.....	A1	50	45	0.00	0.00	0.0
" 3.....	A2	25	50	0.00	0.00	0.0
" 3.....	A3	250	75	0.00	0.00	0.0
" 3.....	A4	75	30	0.00	0.00	0.0
" 3.....	A5	45	40	0.00	0.00	0.0
" 3.....	A6	75	20	0.00	0.00	0.0
Totals.....		520	260			
Aug. 17.....	A1	30	145	3.00	1.50	5.5
" 17.....	A2	300	111	1.75	1.25	5.0
" 17.....	A3	1	x	3.00	1.75	7.0
" 17.....	A4	x	100	2.00	1.10	5.0
" 17.....	A5	300	x	2.50	1.00	7.0
" 17.....	A6	800	x	1.50	1.50	9.5
Totals.....		1,431	356			
Aug. 31.....	A1	700	200	7.50	8.50	0.0
" 31.....	A2	750	150	8.15	9.00	0.0
" 31.....	A3	1	75	6.00	2.50	0.0
" 31.....	A4	1,500	80	6.00	9.50	0.0
" 31.....	A5	1,000	65	2.50	1.50	0.0
" 31.....	A6	2,000	110	8.50	1.50	0.0
Totals.....		5,951	680			

Total nitrites, 1576; total ammonia, 8178.

TABLE XIV.

DATE.	Plot.	Ammonia, parts per million.	Nitrites, parts per million.	Gas production.		
				Glucose bouillon, in cm.	Lactose bouillon, in cm.	Saccharose bouillon, in cm.
Jul. 20.....	A1	25	30	0.00	0.00	0.00
" 20.....	A2	50	30	7.00	1.25	0.00
" 20.....	A3	5	17	7.50	0.00	0.00
" 20.....	A4	50	10	8.50	0.00	0.00
" 20.....	A5	1	15	8.80	0.00	0.00
" 20.....	A6	100	11	10.00	lost	0.00
Totals.....		231	113			
Aug. 3.....	A1	25	15	0.00	0.00	0.00
" 3.....	A2	50	20	0.00	0.00	0.00
" 3.....	A3	25	17	0.00	0.00	0.00
" 3.....	A4	50	20	0.00	0.00	0.00
" 3.....	A5	10	11	3.25	0.00	0.00
" 3.....	A6	90	11	0.00	lost	3.00
Totals.....		250	94			
Aug. 17.....	A1	30	145	3.00	1.50	5.00
" 17.....	A2	300	111	1.75	1.25	7.25
" 17.....	A3	700	x	1.50	1.00	8.00
" 17.....	A4	x	100	2.00	1.50	9.50
" 17.....	A5	lost	x	1.00	1.25	10.00
" 17.....	A6	800	x	1.50	1.00	10.00
Totals.....		1,830	356			
Aug. 31.....	A1	700	150	7.50	8.50	0.00
" 31.....	A2	750	140	8.15	9.90	0.00
" 31.....	A3	500	90	10.50	2.00	0.00
" 31.....	A4	700	40	8.00	1.00	0.00
" 31.....	A5	1,000	75	1.00	.50	0.00
" 31.....	A6	950	30	10.00	9.50	0.00
Totals.....		4,600	525			

Total nitrites, 1088; total ammonia, 6911.

TABLE XV.

DATE.	Plot.	Ammonia, parts per million.	Nitrites, parts per million.	Gas production.		
				Glucose bouillon, in cm.	Lactose bouillon, in cm.	Saccha- rose bouillon, in cm.
Jul. 23.....	B1	25	15	0.00	0.00	0.00
" 23.....	B2	50	27	0.00	2.00	0.00
" 23.....	B3	100	20	4.00	3.00	0.00
" 23.....	B4	50	11	0.00	1.50	1.00
" 23.....	B5	50	12	1.00	5.00	0.00
" 23.....	B6	100	9	1.50	7.00	0.00
Totals.....		375	94			
Aug. 6.....	B1	x	17	4.25	1.75	7.25
" 6.....	B2	x	17	4.00	1.50	8.00
" 6.....	B3	1	10	4.25	1.20	7.50
" 6.....	B4	1	6	0.00	0.00	0.00
" 6.....	B5	1	2	0.00	1.25	0.00
" 6.....	B6	9	8	0.00	.50	7.70
Totals.....		12	60			
Aug. 20.....	B1	100	150	3.25	3.00	0.00
" 20.....	B2	300	145	0.00	0.00	0.00
" 20.....	B3	90	145	3.50	1.25	7.00
" 20.....	B4	400	x	0.00	0.00	0.00
" 20.....	B5	600	120	T	T	0.00
" 20.....	B6	x	75	0.00	0.00	0.00
Totals.....		1,490	635			
Sep. 3.....	B1	2,000	20	11.00	7.00	0.00
" 3.....	B2	0	25	7.00	4.00	0.00
" 3.....	B3	5,000	30	55.00	3.50	0.00
" 3.....	B4	6,000	4	8.50	4.00	0.00
" 3.....	B5	8,000	7	8.50	45.00	0.00
" 3.....	B6	6,000	2	12.00	8.50	0.00
Totals.....		27,000	88			

Total nitrites, 877; total ammonia, 28,877.

TABLE XVI.

DATE.	Plot.	Ammonia, parts per million.	Nitrites, parts per million.	Gas production.		
				Glucose bouillon, in cm.	Lactose bouillon, in cm.	Saccharose bouillon, in cm.
Jul. 23.....	B1	25	10	1.00	0.00	0.00
" 23.....	B2	50	10	1.50	0.00	0.00
" 23.....	B3	75	x	3.50	0.00	0.00
" 23.....	B4	25	5	0.00	0.00	0.00
" 23.....	B5	50	8	0.00	9.00	8.00
" 23.....	B6	100	6	0.00	0.00	1.50
Totals.....		325	39			
Aug. 6.....	B1	x	15	4.25	1.75	7.25
" 6.....	B2	x	16	4.00	1.50	8.00
" 6.....	B3	1	9	4.25	1.00	9.40
" 6.....	B4	4	11	3.75	1.40	8.40
" 6.....	B5	x	4	4.00	1.50	5.75
" 6.....	B6	10	7	4.10	1.00	8.75
Totals.....		15	62			
Aug. 20.....	B1	100	185	3.25	3.00	0.00
" 20.....	B2	300	145	0.00	0.00	0.00
" 20.....	B3	30	145	5.00	0.00	0.00
" 20.....	B4	0	175	0.00	1.00	0.00
" 20.....	B5	8	120	0.00	2.00	0.00
" 20.....	B6	lost	90	0.00	0.00	0.00
Totals.....		438	635			
Sep. 3.....	B1	2,000	20	11.00	7.00	0.00
" 3.....	B2	0	25	7.00	4.00	0.00
" 3.....	B3	6,000	5	8.50	8.50	0.00
" 3.....	B4	8,000	7	8.00	2.00	0.00
" 3.....	B5	8,500	7	9.00	10.00	0.00
" 3.....	B6	9,000	6	8.00	9.50	0.00
Totals.....		33,500	70			

Total nitrites, 1031; total ammonia, 34,278.

NUMBER OF BACTERIA.

Tables V to VIII show a great variation between the number of bacteria at the beginning of the experiment and at the close. This condition holds in all subplots in both sandy and silt loam soils. This may be due to the lessening amount of food available to the bacteria because the temperature, moisture and air remain throughout the experiment within the limit of experimental variation.

Tables V to VIII show exceptionally high numbers of bacteria, sometimes in the surface six inches, and again at the depth of twelve inches. The extra large numbers which are found in some samples from the first six inches of soil were nearly always attributable to manure or quantities of organic matter, while those in the subsoil were usually near some burrow, or at times were due to the presence of a heavy, decaying root.

By comparing tables I to X in the preliminary experiments (part I), it was found that the total number of bacteria in the surface foot of soil is increased by the deeper plowing. This fact is also shown in tables IX to XII (part II), which contain the sum totals of tables V to VIII. In the Marshall silt loam there was also a slight advantage in regard to the total number of bacteria in favor of the plots which were replowed on June 20.

AMMONIA PRODUCTION.

Tables XIII to XVI show the relative amounts of ammonia produced in the different plots. The total amount produced in the silt loam soil exceeded that produced in the sandy loam soil. A comparison of the tables will show that the amount of ammonia produced in the different plots, both in the sandy loam and the silt loam, increased with the depth of plowing. The depth of plowing seems to have greater effect upon the ammonia production in the more tenacious silt soil.

This increase in ammonia production was not regular, but in general conformed to the depth of stirring, although the amount produced varied greatly from week to week. Excess of moisture showed a tendency to lessen the amount of ammonia produced.

Occasionally only a trace of ammonia was present, as will be seen in table XIII, subplot A4, on July 20, and again in subplots A3 and A4, on August 17. This irregularity is found in all the plots. In many cases where these low yields occurred the medium was overgrown with an organism which produced a greenish pigment similar to that of *Ps. fluorescens liquefaciens*. These bacteria seemed to predominate during an excessively moist period.

REDUCTION OF NITRATES.

Denitrification, or the reduction of nitrates to nitrites, with the final liberation of nitrogen gas, may accompany the destructive processes of nitrogen compounds. Some of the ammonia compounds are broken down, liberating free nitrogen, while at the same time the nitrates which may have been formed are reduced to nitrites. These may be broken down into simpler substances, thus releasing free nitrogen.

The results of the experiments testing denitrification are given in tables XIII to XVI, under the heading "Nitrites," ex-

pressed as parts per million. In general, the amount of nitrites produced was in inverse proportion to the amounts of ammonia produced. In general, the production of nitrites decreased as the depth of plowing increased.

SUMMARY.

The results of these preliminary experiments suggest the following conclusions:

1. Deep plowing (eight to ten inches) tends to increase the number of soil bacteria in both sandy and silt soils.

2. Deep plowing tends to increase bacterial activity. More ammonia is produced.

3. Deep plowing tends to decrease denitrification or the reduction of nitrates and the liberation of free nitrogen.

4. The volumetric method of quantitative bacteriological soil analysis has the following possible advantages over the gravimetric method: (a) It is more simple and convenient; (b) there is less danger of contamination; (c) the results are placed on a more accurate basis for comparison. The volumetric method can be used to advantage when comparative results are desired.

5. Increased soil temperature increases bacterial activity.

6. An excess of moisture in soil reduces the number of bacteria and is detrimental to bacterial activity.

7. The maximum number of bacteria is found within the fifth and sixth inches. Either side of this zone the numbers of bacteria decrease.

8. Due to certain conditions, different species of bacteria are present in soil, at different times in predominating numbers.

9. Bacterial life and activity seem to rise and fall with more or less regularity. These periods of maximum and minimum activity are to a certain extent independent of moisture and temperature and are possibly due to the presence of bacterial by-products.

THE FOLLOWING BULLETINS
ARE AVAILABLE FOR DISTRIBUTION TO THOSE RESIDENTS
OF KANSAS WHO MAY DESIRE THEM:

- No. 12....Preliminary Experiments with Fungicides for Stinking Smut of Wheat.
- No. 13....Experiments with Oats.
- No. 15....Additional Experiments and Observation on Oat Smut.
- No. 16....Experiments with Sorghum and with Sugar Beets.
- No. 43....Experiments with Sorghum and with Sugar Beets.
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The Bulletins of the Experiment Station are sent free to applicants. Farmers are invited to send the names of others who would value them and samples will be sent, with return cards upon which applications for future issues may be made.

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